Novel (*R*)-2-Amino-5-fluorotetralins: Dopaminergic Antagonists and Inverse Agonists

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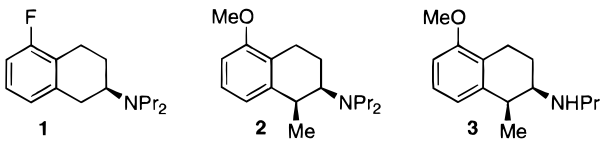
A series of secondary and tertiary *N*-alkyl derivatives of (*R*)-2-amino-5-fluorotetralin have been prepared. The affinities of the compounds for [^{3}H]raclopride-labeled cloned human dopamine (DA) D_2 and D_3 receptors as well as [^{3}H]-8-OH-DPAT-labeled rat hippocampal 5-HT_{1A} receptors were determined. In order to selectively determine affinities for the high-affinity agonist binding site at DA D_2 receptors, the agonist [^{3}H]quinpirole was used. The intrinsic activities of the compounds at DA D_2 and D_3 receptors were evaluated in a [^{35}S]GTPγS binding assay. The novel compounds were characterized as dopaminergic antagonists or inverse agonists. The antagonist (*R*)-2-(butylpropylamino)-5-fluorotetralin (**16**) bound with high affinity (K_i = 4.4 nM) to the DA D_3 receptor and was the most D_3-selective compound (10-fold). (*R*)-2-[[4-(8-Aza-7,9-dioxospiro[4.5]decan-8-yl)butyl]propylamino]-5-fluorotetralin (**18**) bound with very high affinity to both DA D_3 and 5-HT_{1A} receptors (K_i = 0.2 nM) and was also characterized as a dopaminergic antagonist. (*R*)-2-(Benzylpropylamino)-5-fluorotetralin (**10**) behaved as an inverse agonist at both DA D_2 and D_3 receptors. It decreased the basal [^{35}S]GTPγS binding and potently inhibited the DA-stimulated [^{35}S]GTPγS binding. It is apparent that the intrinsic activity of a 2-aminotetralin derivative may be modified by varying the *N*-alkyl substituents.

Introduction

Recently, the concept of inverse agonism (i.e., negative antagonism) and the mechanism by which a ligand activates G protein-coupled (GPC) receptors have been extensively discussed.[1−5] It now appears that the range of efficacies available for a GPC receptor ligand can be expanded from the spectrum defined by that of a fully efficacious agonist over weakly efficacious partial agonists to (neutral/silent) antagonists to include also inverse agonists, i.e., compounds that have the ability to inhibit constitutive receptor signaling. Inverse agonists may become particularly useful as drugs in the therapy of disturbances related to spontaneous, agonist independent GPC receptor overactivity.[6]

In an ongoing project, we study how the efficacy of 2-aminotetralin derivatives at dopamine (DA) D_2 and D_3 receptors can be modified by relatively small structural changes. Although several 2-aminotetralin-derived DA receptor ligands, such as (*S*)-5-hydroxy-2-(dipropylamino)tetralin (5-OH-DPAT),[7] are highly efficacious, various analogues, such as (*R*)-5-F-DPAT (**1**),[8] (*R*)-5-OH-DPAT,[7] (1*S*,2*R*)-UH-232 (**2**),[9] and (1*S*,2*R*)-AJ-76 (**3**),[9] have been characterized as DA D_2 receptor partial agonists, antagonists, or inverse agonists.[7,9,10]



This indicates that the 2-aminotetralin moiety is particularly well suited for studies of the relationship between structure and intrinsic activity.

We now present a series of (*R*)-2-amino-5-fluorotetralin derivatives in which the dopaminergic efficacy has been modified by the introduction of appropriate *N*-substituents; on the basis of their interaction with DA D_2 and D_3 receptors the novel compounds may be characterized as antagonists or inverse agonists. Because many 2-aminotetralin derivatives interact with 5-HT_{1A} receptors,[11−18] we have also measured the affinities for rat 5-HT_{1A} receptors. In fact, most of the novel compounds possess fairly high affinity for 5-HT_{1A} receptors. Hence, the present study and previously established structure−activity relationships[15,19−21] indicate that it will be possible to design 2-aminotetralin derivatives with desired efficacies and affinities for DA D_2/D_3 and 5-HT_{1A} receptors. Such drugs may be of interest as antipsychotic agents.

Chemistry

The synthesis of **5**−**18** is outlined in Scheme 1, and physical data of the novel compounds are given in Table 1. The starting material (*R*)-2-amino-5-fluoro-8-methoxytetralin (**4**)[8] contained trace amounts of 2-amino-8-methoxytetralin, and it was therefore converted into the corresponding *N*-benzyl derivative **5**[14] to facilitate purification. Pure **5** was prepared by reductive alkylation[22] of **4** using benzaldehyde and $NaCNBH_3$ followed by repetitive column chromatography. The amount of 2-(benzylamino)-8-methoxytetralin in purified **5** was determined by GC to be <0.1%. *O*-Demethylation of **5** afforded phenol **6**, which was converted into triflate **7**. Palladium-catalyzed reduction[23] of **7** using Et_3N and formic acid gave **8**. Compound **8** was *N*-debenzylated to give the primary amine **9**, which was either *N*-alkylated with 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione to give **17** or reductively alkylated with acetone and $NaCNBH_3$ to **13**. Reductive alkylation of **8** with propanal or butanal and $NaCNBH_3$ gave **10** and **11** which were *N*-debenzylated to **12** and **14**, respec-

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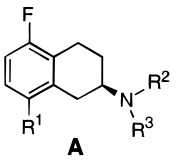
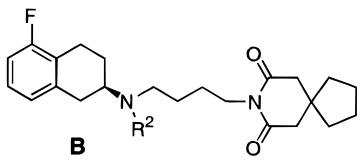
Table 1. Physical Data for Novel Compounds



A

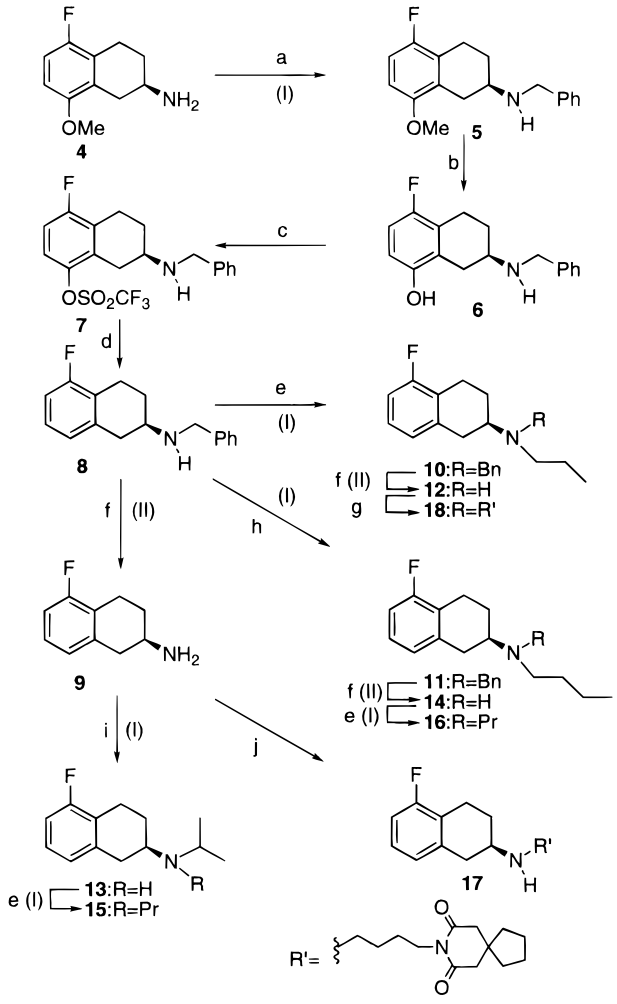


B

compd	structure	R^1	R^2	R^3	method[a]	yield (%)	mp (°C)	recryst solvent[b]	$[\alpha]^{22}_D$ (deg)[c]	formula
6	A	OH	Bn	H	a	99	243−244	A	+54.7	$C_{17}H_{18}FNO\cdot HBr$
7	A	OSO_2CF_3	Bn	H	a	83	232−234	B	+57.7	$C_{18}H_{17}F_4NO_3S\cdot HCl$
8	A	H	Bn	H	a	87	244−246	C	+60.1[d]	$C_{17}H_{18}FN\cdot HCl$
9	A	H	H	H	II	92	273−275	D	+68.7[e]	$C_{10}H_{12}FN\cdot HCl$
10	A	H	Bn	Pr	I	78	100−104	E	+65.6[e]	$C_{20}H_{24}FN\cdot HCl\cdot{}^1/_4H_2O$
11	A	H	Bn	Bu	I	86	139−140	D	+68.3[e]	$C_{21}H_{26}FN\cdot HCl$
12	A	H	Pr	H	II	87	247−250	D	+69.6[e]	$C_{13}H_{18}FN\cdot HCl$
13	A	H	2-Pr	H	I	72	243−244	D	+83.3[f]	$C_{13}H_{18}FN\cdot HCl$
14	A	H	Bu	H	II	91	245[g]	D	+68.1[d]	$C_{14}H_{20}FN\cdot HCl\cdot{}^1/_4H_2O$
15	A	H	Pr	2-Pr	I	20	oil[h]		+54.9[e]	$C_{16}H_{24}FN$[i]
16	A	H	Pr	Bu	I	61	oil[h]		+57.7[j]	$C_{17}H_{26}FN$
17	B		H		a	38	162−163	D	+42.2[f]	$C_{23}H_{31}FN_2O_2\cdot HCl$
18	B		Pr		a	31	oil[h]		+30.4[k]	$C_{26}H_{37}FN_2O_2\cdot{}^1/_2H_2O$

[a] See the Experimental Section. [b] A, MeOH/MeCN; B, 2-PrOH/MeCN; C, EtOH; D, MeOH/ether; E, EtOH/ether. [c] *c* 1.0, MeOH. [d] *c* 0.6. [e] *c* 0.9. [f] *c* 0.8. [g] Decomposition. [h] The HCl salt did not form crystals. Therefore, an analytical sample was prepared of the base. [i] Anal. Calcd for $C_{16}H_{24}FN$: C, 77.1; Found, 76.4. [j] *c* 1.7, $CHCl_3$. [k] *c* 0.14, $CHCl_3$.

Scheme 1[a]



[a] Reagents: (a) PhCHO, $NaCNBH_3$, MeOH; (b) 48% aqueous HBr; (c) $PhN(SO_2CF_3)_2$, K_2CO_3, Et_3N, CH_2Cl_2; (d) $Pd(OAc)_2$, 1,1′-bis(diphenylphosphino)ferrocene (dppf), HCOOH, Et_3N, DMF; (e) EtCHO, $NaCNBH_3$, MeOH; (f) H_2, Pd(C), MeOH; (g) R′-Br, K_2CO_3, MeCN; (h) PrCHO, $NaCNBH_3$, MeOH; (i) $(Me)_2CO$, $NaCNBH_3$, MeOH; (j) R′-Br, KI, K_2CO_3, DMF.

tively. *N*-Alkylation of **12** with 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione gave **18**. Compounds **13** and **14** were reductively alkylated with propanal and $NaCNBH_3$ to afford **15** and **16**, respectively.

Pharmacology

Background. The traditional ternary complex model (TCM) describing ligand−receptor−G protein interactions was presented by the group of Lefkowitz in 1980.[24,25] In this model there is an equilibrium between free receptor (R) and G protein-coupled receptor (RG). The efficacy of a ligand is determined by its affinity ratio for the two forms (R and RG). However, the finding of constitutively active receptors led to an extension of the TCM, the allosteric ternary complex model.[4,26] In this extended model the receptor isomerizes spontaneously between an inactive (R) and an active (R*) conformation, and only the active receptor conformation may interact with the G proteins which in turn leads to a biological response. An agonist stabilizes R*, an inverse agonist stabilizes R, and a neutral antagonist does not affect the equilibrium between these forms. Characteristics of constitutively active systems are (i) an enhanced basal activity, i.e., generation of a signal in the absence of agonist, (ii) an increased affinity for agonists (even in the absence of G protein), (iii) an increased intrinsic activity of partial agonists, and (iv) an ability of some antagonists (inverse agonists), but not all (i.e., neutral or silent antagonists), to inhibit the basal activity (i.e., in the absence of endogenous agonists).[26−34] In systems with low basal activity, inverse agonists and neutral antagonists would be indistinguishable. Thus, the classification of the efficacy of a ligand is system-dependent. A recent study, in which transgenic mice overexpressing myocardial β_2-adrenoceptors were used, provided physiological support for the ternary complex model and the difference in mechanism of action between antagonists and inverse agonists.[35]

Affinity Measurements. The affinity of the compounds for cloned human DA D_2 and D_3 receptors as well as rat hippocampal 5-HT_{1A} receptors was determined using *in vitro* receptor binding studies. The DA receptor antagonist [^{3}H]raclopride was used for labeling of DA D_2 and D_3 receptors.[36] We have previously shown that under the conditions used in the [^{3}H]raclopride assay, i.e., in the presence of sodium ions, only about

Table 2. Affinity of the Compounds Studied to DA D_2, DA D_3, and Serotonin 5-HT_{1A} Receptors

compd	[^{3}H]quinpirole (D_2) K_i (nM)[a]	[^{3}H]raclopride (D_2) K_i (nM)	[^{3}H]raclopride (D_2) n_H[b]	ratio[c]	[^{3}H]raclopride (D_3) K_i (nM)	[^{3}H]raclopride (D_3) n_H[b]	[^{3}H]-8-OH-DPAT (5-HT_{1A}) K_i (nM)
1	26.0 ± 1.7 (3)	201 ± 12 (3)	0.89 ± 0.02	8	8.62 ± 0.63 (3)	1.03 ± 0.05	30.2 ± 3.1 (3)
8	779 ± 147 (2)	2830 ± 120 (3)	0.81 ± 0.02	4	1050 ± 80 (3)	0.88 ± 0.04	494 ± 55 (3)
9	585 ± 47 (2)	864 ± 63 (3)	0.81 ± 0.02	1	150 ± 9 (3)	0.99 ± 0.10	525 ± 17 (3)
10	179 ± 61 (2)	415 ± 41 (3)	0.86 ± 0.06	2	23.8 ± 1.3 (3)	0.92 ± 0.03	28.8 ± 4.0 (3)
12	167 ± 15 (2)	582 ± 30 (3)	0.86 ± 0.02	3	35.8 ± 4.1 (3)	0.92 ± 0.04	108 ± 10 (3)
13	2450 ± 870 (2)	5940 ± 730 (3)	0.88 ± 0.02	2	1450 ± 170 (3)	0.90 ± 0.02	627 ± 63 (3)
14	254 ± 48 (2)	790 ± 31 (3)	0.93 ± 0.06	3	158 ± 7 (3)	0.79 ± 0.06	440 ± 64 (3)
15	1530 ± 190 (2)	4720 ± 60 (3)	0.94 ± 0.05	3	199 ± 18 (3)	0.91 ± 0.06	644 ± 82 (3)
16	45.6 ± 2.5 (2)	210 ± 2 (3)	0.91 ± 0.03	5	4.42 ± 1.14 (3)	0.98 ± 0.10	44.5 ± 4.8 (3)
17	37.0 ± 10.9 (2)	68.2 ± 4.4 (3)	0.88 ± 0.04	2	4.94 ± 0.74 (3)	0.88 ± 0.04	6.46 ± 0.43 (3)
18	4.94 ± 1.85 (2)	16.9 ± 1.3 (3)	0.87 ± 0.09	3	0.251 ± 0.034 (3)	0.92 ± 0.05	0.246 ± 0.036 (3)
dopamine	1.89[d]	572 ± 7 (4)	0.66 ± 0.02	300	13.2 ± 2.3 (4)	0.68 ± 0.02	
haloperidol	0.16[d]	0.66[e]		4	2.74[e]		

[a] The K_i values are means ± standard errors of (n) independent experiments. [b] Hill coefficients are given for [^{3}H]antagonist binding where high- and low-affinity agonist states can be determined. [c] Ratios were calculated from the K_i values determined with [^{3}H]raclopride and [^{3}H]quinpirole for DA D_2 receptors. [d] From ref 37. [e] From ref 36.

Table 3. [^{35}S]GTPγS Binding to Cell Membranes Expressing DA D_2 or D_3 Receptors[a]

compd	D_2 (%)[b]	D_2 +100 μM dopamine[c]	D_3 (%)[b]	D_3 +100 μM dopamine[c]
dopamine	18.8 ± 0.6		19.8 ± 0.2	
1	3.1 ± 1.6	10.5 ± 1.3***	1.6 ± 0.5	18.1 ± 0.1
8	2.7 ± 3.5	15.3 ± 3.8	−8.6 ± 2.1	10.4 ± 0.3**
9	0.8 ± 0.6	14.9 ± 1.3*	−5.0 ± 0.3*	18.4 ± 0.6
10	−12.1 ± 1.5**	−3.4 ± 0.9***	−12.2 ± 0.1**	−6.0 ± 1.4**
12	1.2 ± 1.0	10.7 ± 0.8***	−9.0 ± 0.6*	18.0 ± 3.2
13	0.9 ± 0.7	19.4 ± 1.3	−9.7 ± 0.3*	19.7 ± 2.6
14	−1.1 ± 0.6	10.6 ± 1.6**	−11.2 ± 1.5	18.4 ± 0
15	2.9 ± 2.3	19.5 ± 1.5	0.2 ± 0.1	20.5 ± 2.6
16	1.1 ± 0.3	7.8 ± 0.7***	1.4 ± 2.6	10.6 ± 0.4**
17	−4.1 ± 1.7	0.1 ± 1.6***	−6.5 ± 1.2	9.0 ± 0.7**
18	0.1 ± 1.1	0.8 ± 1.3***	3.1 ± 0.3	9.7 ± 0.4**
haloperidol	−8.3 ± 0.8**	−10.2 ± 0.7***	−19.8 ± 0.1**	2.6 ± 1.6**

[a] The values are given as percent stimulation or inhibition (means ± standard errors of two to four independent experiments) of basal [^{35}]GTPγS binding. [b] The compounds were tested alone (100 μM). An asterisk indicates statistical significance as compared with basal value zero: *p < 0.05, **p < 0.01 (Students t-test). [c] The compounds were tested together with dopamine (100 μM). An asterisk indicates statistical significance as compared with dopamine stimulation: *p < 0.05, **p < 0.01, ***p < 0.001 (Students t-test).

10% of the cloned DA D_2 and D_3 receptors display high affinity for agonists.[37] Therefore, in order to determine the affinity of the compounds for the high-affinity agonist binding site, the agonist [^{3}H]quinpirole was used in conditions promoting high-affinity agonist binding, i.e., in the absence of sodium ions. A selective labeling by [^{3}H]quinpirole of the high-affinity agonist site at the DA D_2 receptor is possible because its affinity for the low-affinity agonist site is too low (about 400 nM) to be measured with the filtration technique.[37] In contrast, the high- and low-affinity agonist sites for [^{3}H]quinpirole at the DA D_3 receptor are too close (about 10-fold separation) for selective labeling of the high-affinity agonist site.[37] In order to determine the affinity of the compounds for 5-HT_{1A} receptors, the 5-HT_{1A}-selective agonist [^{3}H]-8-OH-DPAT was used.[38]

Estimation of Intrinsic Activity. It has previously been shown that the ratio of the affinity constants for the high- and low-affinity agonist sites (K_{low}/K_{high}) correlates with the intrinsic activity of a compound.[24,25,39,40] Thus, a full agonist has a higher ratio than a partial agonist, and an antagonist has a ratio close to 1. We have calculated the ratio between the DA D_2 receptor affinities determined with [^{3}H]raclopride and [^{3}H]quinpirole, respectively, as a measure of intrinsic activity (Table 2). In addition to these indirect measurements, the intrinsic activity of the compounds was directly determined at DA D_2 and D_3 receptors using the [^{35}S]-GTPγS binding method (Table 3).[41−43] When an agonist binds to the receptor, the receptor−G protein interaction is stabilized, which in turn leads to an increased dissociation of GDP from the G protein and GTP (or a nonhydrolyzable GTP analogue, e.g., GTPγS) can bind. Thus, this guanine nucleotide exchange is among the first functional responses in the signal transduction pathway that can be measured after receptor activation. The ability of the compounds to stimulate (agonist properties) or inhibit (inverse agonist properties) the

basal level of [^{35}S]GTPγS binding and also their ability to reverse the DA-induced stimulation of [^{35}S]GTPγS binding (antagonist properties) were determined. In order to be able to measure the responses induced by receptor activation, we included GDP and sodium ions in the assays.[41−43] However, guanine nucleotides and sodium ions reduce the affinity of agonists which makes it necessary to use a high concentration of DA (100 μM).

Results and Discussion

The present series of compounds consists of *N*-mono- and *N*,*N*-dialkylated (*R*)-2-amino-5-fluorotetralin derivatives (Table 1). Among the secondary amines, the *N*-alkyl substituent considerably influenced the receptor binding affinities (Table 2); the introduction of a *N*-benzyl group (**8**) in the primary amine **9** decreased the affinity for the DA D_3 receptors but had little effect on the DA D_2 and the 5-HT_{1A} receptor affinity. The *N*-isopropyl derivative **13** displayed low affinity at the DA D_2 and D_3 receptors, whereas the 5-HT_{1A} receptors appear to better accommodate an *N*-isopropyl group. The introduction of a *N*-butyl group (**14**) in **9** did not alter the affinity at any receptor subtype studied. A *N*-propyl group (**12**) increased the affinity 2−5-fold, whereas the spirocyclic imide derivative **17** dramatically increased the affinity, especially at the DA D_3 and 5-HT_{1A} receptors (30- and 80-fold, respectively).

The introduction of a *N*-propyl group in the secondary amines **8**, **12**, **14**, and **17** increased the affinities for the various receptors. However, the *N*-isopropyl-*N*-propyl derivative **15** had the same affinity as the *N*-isopropyl derivative **13** at the DA D_2 and 5-HT_{1A} receptors. The highest increase in affinity on *N*-propylation was seen at the DA D_3 and 5-HT_{1A} receptors; **10**, **16**, and **18** had 44-, 36-, and 20-fold higher affinity at the DA D_3 receptor and 14-, 10-, and 26-fold higher affinity at the 5-HT_{1A} receptor as compared to the secondary amines. A comparison of DA D_2 receptor affinities shows that the increase was only 4−7-fold. This indicates that a propyl group contributes more to the binding energy at the DA D_3 and 5-HT_{1A} receptors than at the DA D_2 receptor. Thus, the fit of the propyl group in the receptor cavity of the DA D_3 and 5-HT_{1A} receptors appears to be better. These results agree with a previous study where we described a "propyl cleft" in the DA D_2 and D_3 receptor models and speculated that the DA D_3 receptor seems to be more sensitive to the length of the *N*-substituent than the DA D_2 receptor.[21] Generally, the DA D_2 receptor appears to be less sensitive to modifications of the *N*-alkyl substituents.

The affinity of the compounds for DA D_2 receptors was determined using two different radioligands in different assay conditions: The [^{3}H]quinpirole assay was used for the high-affinity agonist binding site and the [^{3}H]-raclopride assay for the low-affinity agonist binding site.[37] The [^{3}H]raclopride(D_2)/[^{3}H]quinpirole(D_2) ratios ranged between 1 and 8, indicating low intrinsic activities of the compounds.[10,40] These ratios may be compared with those of the endogenous DA (300) and the DA D_2 receptor antagonist haloperidol (4) (Table 2). The low agonist activities of the novel compounds were corroborated by their DA D_2 and D_3 receptor-mediated effects on basal [^{35}S]GTPγS binding (Table 3). Interestingly, the *N*-benzyl-*N*-propyl derivative **10** (ASA-6) reduced the basal level of [^{35}S]GTPγS binding, i.e., it behaved as an inverse agonist at the DA D_2 receptor. Haloperidol also showed inverse agonism as suggested previously.[44] Both **10** and haloperidol were also able to inhibit the DA-induced stimulation of [^{35}S]GTPγS binding, and it is well known that DA does not affect cells transfected with plasmid but lacking receptor cDNA. Furthermore, pertussis toxin treatment of the cells abolished both the DA-induced stimulation and the haloperidol-induced inhibition of [^{35}S]GTPγS binding (unpublished results). The above experimental data provide support for the notion that the inverse agonism of **10** is due to a receptor-mediated activation of G_i/G_o proteins.

Compounds **17** and **18** did not display any intrinsic activity, and both were potent antagonists at the DA D_2 receptor (i.e., they inhibited DA-stimulated [^{35}S]-GTPγS binding to the basal level). The *N*-propyl and *N*-butyl derivatives **1**, **12**, **14**, and **16**, as well as the primary amine **9**, were antagonists, although not of the same potency as **17** and **18**. The low potencies were probably due to the low affinities for the DA D_2 receptor. The other compounds in this series (**8**, **13**, and **15**) had low affinities at the DA D_2 receptor and were inactive in the [^{35}S]GTPγS binding assay.

The intrinsic activities of the compounds at DA D_3 receptors were also tested in the [^{35}S]GTPγS binding assay. However, the functional coupling of the DA D_3 receptor is not well established. Compared with DA D_2 receptor activation, less efficacious second-messenger coupling and agonist-induced stimulation have been reported for DA D_3 receptors.[45−48] Thus, due to the uncertainties in the functional coupling of DA D_3 receptors, one should be cautious in drawing definite conclusions from the results in Table 3. It is noteworthy, however, that the *N*-benzyl-*N*-propyl derivative **10** and haloperidol behaved as inverse agonists not only at the DA D_2 receptor but also at the DA D_3 receptor (Table 3). Compounds **16**−**18** did not exhibit any intrinsic activity at the DA D_3 receptor, but they were able to inhibit the DA-induced stimulation. Surprisingly, the *N*-benzyl derivative **8** did inhibit the stimulation induced by DA despite its low DA D_3 receptor affinity.

Agonists have a small separation between the high- and low-affinity sites at the DA D_3 receptor (about 10-fold) in contrast to their behavior at the DA D_2 receptor.[37,45,49] Due to the inclusion of sodium ions and GDP in the [^{35}S]GTPγS binding assay, the DA D_2 and D_3 receptors probably are in their low affinity state for agonists. Consequently, DA binds with relatively high affinity to the DA D_3 receptor in this assay compared to its binding to DA D_2 receptors. This might be a possible explanation for the difficulty of some antagonists to inhibit the DA-induced stimulation.

A larger number of compounds behaved as inverse agonists at the DA D_3 receptor than at the DA D_2 receptor. This might be due to the higher receptor density (3.5 pmol/mg of protein for D_3 and 1 pmol/mg of protein for D_2) in the cell line expressing DA D_3 receptors[34] and/or the expression of receptors in different cell lines (CHO and Ltk$^-$ cells, respectively). An alternative explanation may be that the DA D_3 receptor is more closely associated with the G protein than the DA D_2 receptor. Such a close association might lead to a higher basal activity, and observations of inverse

agonism would thereby be easier. Even though the characterization of the intrinsic properties of a ligand is tissue-dependent, a comparison of efficacies between ligands within a specific system is still valid.

The intrinsic activity of a ligand is not dependent on the affinity. This is illustrated by comparing the high-affinity spirocyclic imide derivative **18** (antagonist) with the *N*-benzyl-*N*-propyl derivative **10** (inverse agonist). Compound **10** has 40-fold lower affinity at the DA D_2 receptor and 95-fold lower affinity at the DA D_3 receptor compared to **18**. Despite this affinity difference, **10** is able to inhibit basal activity, whereas **18** is not. Furthermore, at the DA D_3 receptor, **10** is the only compound that potently blocks the DA-induced stimulation. The effect of the *N*-benzyl group on the intrinsic activity is illustrated by comparing the *N*-propyl derivative **12** and the *N*-benzyl-*N*-propyl derivative **10**. Compounds **10** and **12** had almost identical affinity at the DA D_2 (582 and 415 nM, respectively, [^{3}H]raclopride binding) and DA D_3 (36 and 24 nM, respectively) receptors. Compound **10**, however, showed inverse agonism at both receptors, whereas **12** behaved as an antagonist at the DA D_2 receptor and was unable to inhibit the DA stimulation at the DA D_3 receptor. Consequently, the *N*-benzyl group appears to contribute to inverse agonism without affecting the affinity.

It is noteworthy that the affinity (K_i value) and the potency of inhibiting DA-stimulated [^{35}S]GTPγS binding cannot directly be compared due to the presence of GDP in the functional assay. The presence of guanine nucleotides is known to decrease the binding affinity of agonists,[24,25,37] but their effect on the binding of inverse agonists has not yet been established. According to the extended ternary complex model, guanine nucleotides would increase the affinity of inverse agonists rather than decrease it.[4]

In conclusion, the present series of (*R*)-2-amino-5-fluorotetralin derivatives includes inverse agonists and antagonists. Thus, it is possible to change the intrinsic activity by varying the *N*-alkyl substituents of (*R*)-2-amino-5-fluorotetralin. It is also apparent that the secondary amines generally have lower intrinsic activity than the tertiary amines (cf. also **2** and **3**), with the exception of the *N*-benzyl derivative. Several compounds display high 5-HT_{1A} receptor affinity, and future studies will address their efficacies at this receptor. It should be noted that **10** (ASA-6) is the first reported inverse agonist with DA D_3 versus D_2 selectivity. Despite its moderate affinity, **10** showed pronounced inverse agonism at both DA D_2 and D_3 receptors. The pharmacological profile of this compound will be further examined.

Experimental Section

Chemistry. General Comments. Routine ^{1}H and ^{13}C NMR spectra were recorded at 270 and 67 MHz, respectively, on a JEOL EX-270 spectrometer and were referenced to internal tetramethylsilane. ^{1}H and ^{13}C NMR spectra for compound **18** was recorded at 400 and 100 MHz, respectively, on a Varian Unity 400 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 Series FTIR spectrometer. All spectra were in accordance with the assigned structures. Melting points (uncorrected) were determined in open glass capillaries on an Electrothermal melting point apparatus. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. The optical rotation for compound **18** was obtained on a POLAAR 2000 AA Series polarimeter. The elemental analyses (C, H, and N) were performed by Mikro Kemi AB, Uppsala, Sweden, or by Analytische Laboratorien, Lindlar, Germany. The found values were within 0.4% of the theoretical values if not otherwise stated. Capillary GC analyses were performed on a Shimadzu GC-14A instrument equipped with a HP-1 (50 m × 0.32 mm) column or on a Carlo Erba 4160 capillary GC instrument equipped with a 0.25 μM CP-Sil-5 (25 m × 0.25 mm) column (compound **18**). Low-resolution electron-impact mass spectral data (70 eV) were obtained on a Hewlett-Packard mass spectrometer (HP5971A MSD) connected to a gas chromatograph (HP GC5890 Series 2), equipped with a HP-1 (25 m × 0.32 mm) column, or a JEOL mass spectrometer (Automass System II) connected to a Hewlett-Packard HP6890 Series equipped with a 0.12 μM CP-Sil-5 (25 m × 0.25 mm) column (compound **18**). Column chromatography was performed on silica gel 60 (0.04−0.063 or 0.015-0.04 mm; Merck) or aluminum oxide 90 (0.063−0.200 mm; Merck). Thin layer chromatography was performed on aluminum sheets precoated with either silica gel 60 F254 or aluminum oxide 60 F254 E neutral (0.2 mm; Merck).

(*R*)-2-(Benzylamino)-5-fluoro-8-methoxytetralin Hydrochloride (5·HCl). Method I. A mixture of **4** (1.76 g, 9.01 mmol), containing trace amounts of 2-amino-8-methoxytetralin, and benzaldehyde (2.87 g, 27.0 mmol) in MeOH (60 mL) was stirred under N_2 at room temperature for 2 h. The pH was adjusted to 5 by addition of HOAc. The reaction mixture was cooled (ice bath), and $NaCNBH_3$ (1.70 g, 27.0 mmol) was added. After 4 h at room temperature the solvent was removed under reduced pressure, and the residue was partitioned between 10% aqueous Na_2CO_3 and ether. The organic phase was dried (K_2CO_3), filtered, and concentrated. The crude oil was purified by repetitive chromatography [SiO_2, MeOH/CH_2Cl_2 (1:39) saturated with NH_3]. The amine was converted into the hydrochloride salt, which was recrystallized to afford 1.72 g of pure **5**·HCl: mp 277−280 °C (lit.[50] mp 281−282 °C); $[\alpha]^{22}_D$ +60.8° (*c* 1.0, MeOH) [lit.[50] $[\alpha]^{22}_D$ +62.0° (*c* 1.0, MeOH)]; ^{1}H NMR (CD_3OD) δ 1.74−1.90 (m, 1 H), 2.39−2.47 (m, 1 H), 2.60−2.80 (m, 2 H), 3.03−3.10 (m, 1 H), 3.36−3.44 (m, 1 H), 3.50−3.62 (m, 1 H), 3.82 (s, 3 H), 4.36 (s, 2 H), 6.77−6.81 (m, 1 H), 6.88−6.94 (m, 1 H), 7.45−7.58 (m, 5 H); ^{13}C NMR (CD_3OD) δ 22.1 (d, $^3J_{C,F}$ = 3.7 Hz), 25.8, 27.7, 49.8, 55.6, 56.2, 109.3 (d, $^3J_{C,F}$ = 8.5 Hz), 113.6 (d, $^2J_{C,F}$ = 23.2 Hz), 123.8 (d, $^3J_{C,F}$ = 4.9 Hz), 124.6 (d, $^2J_{C,F}$ = 19.5 Hz), 130.4 (2C:s), 130.7, 131.0 (2C:s), 132.9, 154.6, 156.2 (d, $^1J_{C,F}$ = 235.6 Hz).

(*R*)-2-(Benzylamino)-5-fluoro-8-hydroxytetralin Hydrobromide (6·HBr). A stirred mixture of **5**·HCl (3.37 g, 10.5 mmol) and aqueous 48% HBr (100 mL) was refluxed (120 °C) under N_2 for 7 h. The volatiles were evaporated under reduced pressure to give a solid residue which was recrystallized to afford 3.68 g of pure **6**·HBr: ^{1}H NMR (CD_3OD) δ 1.74−1.89 (m, 1 H), 2.40−2.47 (m, 1 H), 2.59−2.78 (m, 2 H), 3.00−3.08 (m, 1 H), 3.34−3.44 (m, 1 H), 3.51−3.63 (m, 1 H), 4.37 (s, 2 H), 6.58−6.63 (m, 1 H), 6.72−6.79 (m, 1 H), 7.45−7.59 (m, 5 H); ^{13}C NMR (CD_3OD) δ 22.1 (d, $^3J_{C,F}$ = 3.6 Hz), 25.9, 27.8, 49.9, 55.7, 113.3 (d, $^3J_{C,F}$ = 8.5 Hz), 113.6 (d, $^2J_{C,F}$ = 23.2 Hz), 122.1 (d, $^3J_{C,F}$ = 3.6 Hz), 124.2 (d, $^2J_{C,F}$ = 19.6 Hz), 130.3 (2C:s), 130.7, 131.1 (2C:s), 132.8, 152.2, 155.4 (d, $^1J_{C,F}$ = 233.1 Hz).

(*R*)-2-(Benzylamino)-5-fluoro-8-[[(trifluoromethyl)sulfonyl]oxy]tetralin Hydrochloride (7·HCl). A mixture of **6**·HBr (3.45 g, 9.78 mmol), K_2CO_3 (5.41 g, 39.1 mmol), and Et_3N (5.45 mL, 39.1 mmol) in CH_2Cl_2 (50 mL) was refluxed (40 °C) under N_2 for 1 h. *N*-Phenyltrifluoromethanesulfonimide (7.34 g, 20.5 mmol) was added, and the reaction mixture was refluxed for 22 h, filtered, and concentrated under reduced pressure. The crude oil was purified by column chromatography (SiO_2, CH_2Cl_2). The amine was converted into the hydrochloride salt, which was recrystallized to afford 3.58 g of **7**·HCl: ^{1}H NMR (CD_3OD) δ 1.85−1.98 (m, 1 H), 2.51−2.56 (m, 1 H), 2.79−2.95 (m, 2 H), 3.12−3.18 (m, 1 H), 3.42−3.50 (m, 1 H), 3.60−3.70 (m, 1 H), 4.29−4.44 (m, 2 H), 7.13−7.19 (m, 1 H), 7.27−7.32 (m, 1 H), 7.47−7.58 (m, 5 H); ^{13}C NMR (CD_3OD) δ 22.0 (d, $^3J_{C,F}$ = 3.6 Hz), 24.9, 28.2, 50.1, 54.5, 115.5 (d, $^2J_{C,F}$ = 24.4 Hz), 120.0 (q, $^1J_{C,F}$ = 319.8 Hz), 121.8 (d, $^3J_{C,F}$ = 9.8 Hz), 127.2 (d, $^2J_{C,F}$ = 20.7 Hz), 129.7 (d, $^3J_{C,F}$ = 4.9 Hz), 130.4 (2C:s), 130.8, 131.1 (2C:s), 132.6, 144.8 (d, $^4J_{C,F}$ = 2.4 Hz), 160.8 (d, $^1J_{C,F}$ = 246.6 Hz).

(*R*)-2-(Benzylamino)-5-fluorotetralin Hydrochloride (8·HCl). A mixture of **7** (3.17 g, 7.86 mmol), Et_3N (3.28 mL, 23.6 mmol), 1,1′-bis(diphenylphosphino)ferrocene (dppf) (435 mg, 0.79 mmol), $Pd(OAc)_2$ (88 mg, 0.39 mmol), and concentrated formic acid (0.60 mL, 15.7 mmol) in DMF (50 mL) was stirred under N_2 at 60 °C for 2 h. The mixture was partitioned between brine, saturated Na_2CO_3, and ether. The organic phase was dried (K_2CO_3), filtered, and concentrated. The residue was chromatographed [SiO_2, MeOH/CH_2Cl_2 (1:79) saturated with NH_3]. The amine was converted into the hydrochloride salt, which was recrystallized to afford 2.00 g of pure **8**·HCl: ^{1}H NMR (CD_3OD) δ 1.80−1.96 (m, 1 H), 2.43−2.50 (m, 1 H), 2.71−2.84 (m, 1 H), 2.93−3.12 (m, 2 H), 3.35−3.43 (m, 1 H), 3.54−3.65 (m, 1 H), 4.36 (s, 2 H), 6.88−6.95 (m, 1 H), 6.98−7.01 (m, 1 H), 7.14−7.22 (m, 1 H), 7.46−7.59 (m, 5 H); ^{13}C NMR (CD_3OD) δ 21.7 (d, $^3J_{C,F}$ = 4.9 Hz), 26.2, 32.8, 50.0, 55.5, 113.9 (d, $^2J_{C,F}$ = 21.9), 123.6 (d, $^2J_{C,F}$ = 18.3 Hz), 125.9 (d, $^3J_{C,F}$ = 3.6 Hz), 128.8 (d, $^3J_{C,F}$ = 8.5 Hz), 130.4 (2C:s), 130.7, 131.1 (2C:s), 132.8, 136.1 (d, $^3J_{C,F}$ = 3.7 Hz), 162.1 (d, $^1J_{C,F}$ = 244.1 Hz).

(*R*)-2-(Benzylpropylamino)-5-fluorotetralin Hydrochloride (10·HCl). Compound **10** was prepared from **8** (653 mg, 2.56 mmol), propanal (1.84 mL, 25.6 mmol), and $NaCNBH_3$ (1.61 g, 25.6 mmol) using method I. The reaction time was 44 h. The residue was purified by chromatography [SiO_2, ether/petroleum ether (1:9) saturated with NH_3]. The amine was converted into the hydrochloride salt to give 662 mg of **10**·HCl. An analytical sample of **10**·HCl was prepared by recrystallization: ^{1}H NMR (CD_3OD) δ 0.94 (t, 3 H, J = 7.3 Hz), 1.50−1.92 (m, 2 H), 1.96−2.12 (m, 1 H), 2.40−2.53 (m, 1 H), 2.68−2.81 (m, 1 H), 3.13−3.31 (m, 5 H), 3.70−3.80 (m, 1 H), 4.42−4.59 (m, 2 H), 6.88−6.95 (m, 1 H), 7.01−7.03 (m, 1 H), 7.14−7.22 (m, 1 H), 7.49−7.61 (m, 5 H); ^{13}C NMR (CD_3OD) δ 11.3, 19.5, 22.6 (d, $^3J_{C,F}$ = 4.9 Hz), 24.2, 30.3, 53.0, 56.0, 61.0, 113.8 (d, $^2J_{C,F}$ = 22.0 Hz), 123.5 (d, $^2J_{C,F}$ = 18.3 Hz), 126.1 (d, $^4J_{C,F}$ = 2.5 Hz), 128.8 (d, $^3J_{C,F}$ = 8.5 Hz), 130.5 (2C:s), 131.2, 131.4, 132.2 (2C:s), 136.6 (d, $^3J_{C,F}$ = 4.9 Hz), 162.0 (d, $^1J_{C,F}$ = 244.1 Hz).

(*R*)-2-(Benzylbutylamino)-5-fluorotetralin Hydrochloride (11·HCl). Compound **11** was prepared from **8** (276 mg, 1.08 mmol), butanal (0.97 mL, 10.8 mmol), and $NaCNBH_3$ (679 mg, 10.8 mmol) using method I. Additional butanal (0.292 mL, 3.24 mmol) was added after 5 days, and the reaction mixture was heated at 40 °C. After a total of 6 days the reaction was interrupted. The residue was chromatographed [SiO_2, ether/petroleum ether (1:9) saturated with NH_3]. The amine was converted into the hydrochloride salt to give 308 mg of **11**·HCl. An analytical sample of **11**·HCl (40 mg) was prepared by recrystallization: ^{1}H NMR (CD_3OD) δ 0.92 (t, 3 H, J = 7.3 Hz), 1.27−1.40 (m, 2 H), 1.50−1.80 (m, 2 H), 1.96−2.12 (m, 1 H), 2.42−2.52 (m, 1 H), 2.68−2.81 (m, 1 H), 3.11−3.32 (m, 5 H), 3.70−3.82 (m, 1 H), 4.35−4.63 (m, 2 H), 6.88−6.95 (m, 1 H), 7.01−7.03 (m, 1 H), 7.14−7.22 (m, 1 H), 7.49−7.55 (m, 3 H), 7.59−7.64 (m, 2 H); ^{13}C NMR (CD_3OD) δ 13.8, 21.0, 22.6 (d, $^3J_{C,F}$ = 3.6 Hz), 24.3, 28.0, 30.4, 51.3, 56.0, 61.1, 113.8 (d, $^2J_{C,F}$ = 20.7 Hz), 123.6 (d, $^2J_{C,F}$ = 18.3 Hz), 126.1 (d, $^4J_{C,F}$ = 2.5 Hz), 128.7 (d, $^3J_{C,F}$ = 8.5 Hz), 130.5 (2C:s), 131.1, 131.7, 132.2 (2C:s), 136.7 (d, $^3J_{C,F}$ = 4.9 Hz), 162.0 (d, $^1J_{C,F}$ = 244.1 Hz).

(*R*)-5-Fluoro-2-(2-propylamino)tetralin Hydrochloride (13·HCl). Compound **13** was prepared from **9** (214 mg, 1.29 mmol), acetone (0.67 mL, 9.07 mmol), MeOH (5 mL), and $NaCNBH_3$ (570 mg, 9.07 mmol) using method I. The reaction time was 24 h. The crude oil was chromatographed [SiO_2, MeOH/CH_2Cl_2 (1:9) saturated with NH_3] to yield 222 mg of **13**. A small sample of **13** was converted into the hydrochloride salt and recrystallized to give **13**·HCl: ^{1}H NMR (CD_3OD) δ 1.39 (d, 3 H, J = 6.4 Hz), 1.40 (d, 3 H, J = 6.5 Hz), 1.75−1.90 (m, 1 H), 2.32−2.40 (m, 1 H), 2.73−2.95 (m, 2 H), 3.01−3.13 (m, 1 H), 3.25−3.33 (m, 1 H), 3.58−3.71 (m, 2 H), 6.88−6.95 (m, 1 H), 6.97−7.00 (m, 1 H), 7.13−7.21 (m, 1 H); ^{13}C NMR (CD_3OD) δ 19.3, 19.7, 21.7 (d, $^3J_{C,F}$ = 4.9 Hz), 26.4, 32.8 (d, $^4J_{C,F}$ = 2.4 Hz), 48.6, 52.1, 113.8 (d, $^2J_{C,F}$ = 21.9 Hz), 123.7 (d, $^2J_{C,F}$ = 18.3 Hz), 125.9 (d, $^4J_{C,F}$ = 3.7 Hz), 128.7 (d, $^3J_{C,F}$ = 8.5 Hz), 136.2 (d, $^3J_{C,F}$ = 4.9 Hz), 162.0 (d, $^1J_{C,F}$ = 244.2 Hz).

(*R*)-5-Fluoro-2-[(2-propyl)propylamino]tetralin (15). Compound **15** was prepared from **13** (119 mg, 0.574 mmol), propanal (0.50 mL, 6.89 mmol), MeOH (5 mL), and $NaCNBH_3$ (433 mg, 6.89 mmol) using method I. After reflux (40 °C) for 7 days propanal (0.40 mL, 5.74 mmol), $NaCNBH_3$ (360 mg, 5.73 mmol), and MeOH (2 mL) were added. After a total of 15 days the reaction was interrupted. The crude oil was purified by column chromatography [SiO_2, MeOH/CH_2Cl_2 (1:39) saturated with NH_3] followed by preparative TLC (Al_2O_3, petroleum ether) to give 29 mg of **15**: ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H, J = 7.4 Hz), 1.05 (d, 6 H, J = 6.5 Hz), 1.37−1.50 (m, 2 H), 1.53−1.69 (m, 1 H), 1.98−2.08 (m, 1 H), 2.38−2.56 (m, 2 H), 2.56−2.69 (m, 1 H), 2.77−2.81 (m, 2 H), 2.93−3.19 (m, 3 H), 6.76−6.86 (m, 2 H), 7.01−7.09 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 11.7, 20.4, 20.9, 22.8 (d, $^3J_{C,F}$ = 3.7 Hz), 24.4, 27.2, 34.5, 47.6, 48.6, 54.0, 111.7 (d, $^2J_{C,F}$ = 22.0 Hz), 123.9 (d, $^2J_{C,F}$ = 17.1 Hz), 124.7 (d, $^4J_{C,F}$ = 3.7 Hz), 126.4 (d, $^3J_{C,F}$ = 8.5 Hz), 140.0 (d, $^3J_{C,F}$ = 4.9 Hz), 160.9 (d, $^1J_{C,F}$ = 242.9 Hz).

(*R*)-2-(Butylpropylamino)-5-fluorotetralin (16). Compound **16** was prepared from **14** (89 mg, 0.40 mmol), propanal (0.35 mL, 4.83 mmol), MeOH (5 mL), and $NaCNBH_3$ (303 mg, 4.83 mmol) using method I. After 7 days additional propanal (0.14 mL, 2.01 mmol) and $NaCNBH_3$ (126 mg, 2.01 mmol) were added, and after 2 more days the reaction was interrupted. The residue was chromatographed [SiO_2, MeOH/CH_2Cl_2 (1:49) saturated with NH_3 followed by Al_2O_3, ether/petroleum ether (1:19)] to give 65 mg of **16**: ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3 H, J = 7.4 Hz), 0.91 (t, 3 H, J = 7.3 Hz), 1.27−1.64 (m, 7 H), 2.01−2.10 (m, 1 H), 2.44−2.53 (m, 4 H), 2.55−3.06 (m, 5 H), 6.77−6.83 (m, 1 H), 6.85−6.88 (m, 1 H), 6.98−7.09 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 11.9, 14.1, 20.7, 22.2, 22.8 (d, $^3J_{C,F}$ = 3.6 Hz), 25.0, 31.3, 32.0 (d, $^4J_{C,F}$ = 2.4 Hz), 50.3, 52.5, 56.2, 111.8 (d, $^2J_{C,F}$ = 21.9 Hz), 124.0 (d, $^2J_{C,F}$ = 17.1 Hz), 124.7 (d, $^4J_{C,F}$ = 2.4 Hz), 126.4 (d, $^3J_{C,F}$ = 8.5 Hz), 139.6 (d, $^3J_{C,F}$ = 3.6 Hz), 160.8 (d, $^1J_{C,F}$ = 242.9 Hz).

(*R*)-5-Fluoro-2-(propylamino)tetralin Hydrochloride (12·HCl). Method II. A mixture of **10**·HCl (586 mg, 1.76 mmol), Pd(C) (10%, 0.5 g), and MeOH (30 mL) was hydrogenated at atmospheric pressure for 25 h. The mixture was diluted with CH_2Cl_2/MeOH (4:1) and filtered through Celite. The volatiles were evaporated under reduced pressure, and the crude product was recrystallized to give 372 mg of **12**·HCl: ^{1}H NMR (CD_3OD) δ 1.07 (t, 3 H, J = 7.4 Hz), 1.71−1.91 (m, 3 H), 2.33−2.44 (m, 1 H), 2.71−2.84 (m, 1 H), 2.87−2.97 (m, 1 H), 3.00−3.14 (m, 3 H), 3.27−3.36 (m, 1 H), 3.47−3.58 (m, 1 H), 6.88−6.94 (m, 1 H), 6.97−7.00 (m, 1 H), 7.13−7.21 (m, 1 H); ^{13}C NMR (CD_3OD) δ 11.4, 21.0, 21.6 (d, $^3J_{C,F}$ = 3.7 Hz), 26.2, 32.7 (d, $^4J_{C,F}$ = 2.4 Hz), 47.9, 55.4, 113.8 (d, $^2J_{C,F}$ = 22.0 Hz), 123.6 (d, $^2J_{C,F}$ = 18.3 Hz), 125.9 (d, $^4J_{C,F}$ = 3.6 Hz), 128.7 (d, $^3J_{C,F}$ = 8.6 Hz), 136.1 (d, $^3J_{C,F}$ = 4.9 Hz), 162.0 (d, $^1J_{C,F}$ = 242.9 Hz).

(*R*)-2-Amino-5-fluorotetralin Hydrochloride (9·HCl). Compound **9**·HCl was prepared from **8**·HCl (800 mg, 2.74 mmol), Pd(C) (10%, 0.5 g), and MeOH (250 mL) using method II. After 20 h the reaction was interrupted. The crude product was recrystallized to give 511 mg of **9**·HCl: ^{1}H NMR (CD_3OD) δ 1.78−1.93 (m, 1 H), 2.26−2.32 (m, 1 H), 2.72−3.06 (m, 3 H), 3.19−3.26 (m, 1 H), 3.50−3.61 (m, 1 H), 6.87−6.97 (m, 2 H), 7.12−7.20 (m, 1 H); ^{13}C NMR (CD_3OD) δ 21.3 (d, $^3J_{C,F}$ = 3.7 Hz), 27.4, 34.1, 48.4, 113.8 (d, $^2J_{C,F}$ = 20.8 Hz), 123.5 (d, $^2J_{C,F}$ = 18.3 Hz), 125.9 (d, $^4J_{C,F}$ = 2.4 Hz), 128.6 (d, $^3J_{C,F}$ = 8.6 Hz), 136.2 (d, $^3J_{C,F}$ = 3.7 Hz), 162.1 (d, $^1J_{C,F}$ = 244.1 Hz).

(*R*)-2-(Butylamino)-5-fluorotetralin Hydrochloride (14·HCl). Compound **14**·HCl was prepared from **11**·HCl (278 mg, 0.80 mmol), Pd(C) (10%, 0.5 g), and MeOH (15 mL) using method II. After 24 h the reaction was interrupted. The crude product was recrystallized to give 188 mg of **14**·HCl: ^{1}H NMR (CD_3OD) δ 1.02 (t, 3 H, J = 7.3 Hz), 1.48 (sext, 2 H, J = 7.4 Hz), 1.67−1.91 (m, 3 H), 2.34−2.44 (m, 1 H), 2.71−2.84 (m, 1 H), 2.87−2.97 (m, 1 H), 3.00−3.17 (m, 3 H), 3.28−3.36 (m, 1 H), 3.47−3.58 (m, 1 H), 6.88−6.94 (m, 1 H), 6.97−7.00 (m, 1 H), 7.13−7.21 (m, 1 H); ^{13}C NMR (CD_3OD) δ 14.0, 20.9, 21.6 (d, $^3J_{C,F}$ = 4.9 Hz), 26.2, 29.5, 32.7 (d, $^4J_{C,F}$ = 2.4 Hz), 46.1, 55.4, 113.8 (d, $^2J_{C,F}$ = 21.9 Hz), 123.6 (d, $^2J_{C,F}$ = 18.3 Hz), 125.9 (d, $^4J_{C,F}$ = 2.4 Hz), 128.7 (d, $^3J_{C,F}$ = 9.7 Hz), 136.1 (d, $^3J_{C,F}$ = 4.9 Hz), 162.0 (d, $^1J_{C,F}$ = 242.9 Hz).

(*R*)-2-[[4-(8-Aza-7,9-dioxospiro[4.5]decan-8-yl)butyl]amino]-5-fluorotetralin Hydrochloride (17·HCl). A mixture

of **9** (116 mg, 0.70 mmol), 8-(4-bromobutyl)-8-azaspiro[4.5]-decane-7,9-dione[8,51] (233 mg, 0.77 mmol), K_2CO_3 (388 mg, 2.81 mmol), and KI (1.2 mg, 7 μmol) in DMF (1.5 mL) was stirred under N_2 for 23 h at room temperature. The mixture was diluted with ether, filtered, and concentrated. The residue was chromatographed [SiO_2, MeOH/CH_2Cl_2 (1:49) saturated with NH_3], and the amine was converted into the hydrochloride salt, which was recrystallized to afford 113 mg of **17**·HCl: IR (neat) 1670 cm^{-1} (C═O); ^1H NMR (CD_3OD) δ 1.49−1.54 (m, 4 H), 1.63−1.91 (m, 9 H), 2.37−2.41 (m, 1 H), 2.66 (s, 4 H), 2.71−2.84 (m, 1 H), 2.85−2.95 (m, 1 H), 3.03−3.09 (m, 1 H), 3.15−3.21 (m, 2 H), 3.28−3.35 (m, 1 H), 3.46−3.57 (m, 1 H), 3.81 (t, 2 H, J = 6.7 Hz), 6.88−6.95 (m, 1 H), 6.97−7.00 (m, 1 H), 7.13−7.21 (m, 1 H); ^{13}C NMR (CD_3OD) δ 21.5 (d, $^3J_{C,F}$ = 4.7 Hz), 24.9, 25.1 (2C:s), 26.1 (2C:s), 32.7 (d, $^4J_{C,F}$ = 2.1 Hz), 38.4 (2C:s), 39.4, 40.6, 45.4 (2C:s), 45.9, 55.4, 113.8 (d, $^2J_{C,F}$ = 21.8 Hz), 123.6 (d, $^2J_{C,F}$ = 18.0 Hz), 125.9 (d, $^4J_{C,F}$ = 3.1 Hz), 128.7 (d, $^3J_{C,F}$ = 8.4 Hz), 136.1 (d, $^3J_{C,F}$ = 4.8 Hz), 162.0 (d, $^1J_{C,F}$ = 243.5 Hz), 174.5 (2C:s).

(*R*)-2-[[4-(8-Aza-7,9-dioxospiro[4.5]decan-8-yl)butyl]propylamino)-5-fluorotetralin (18). A stirred mixture of **12**·HCl (110 mg, 0.45 mmol), 8-(4-bromobutyl)-8-azaspiro[4.5]-decane-7,9-dione (177 mg, 0.59 mmol), and K_2CO_3 (249 mg, 1.80 mmol) in MeCN (1.5 mL) was refluxed (80 °C) under N_2 for 3 days. The mixture was diluted with ether, filtered, and concentrated. The residue was chromatographed [SiO_2, MeOH/CH_2Cl_2 (1:49) saturated with NH_3 followed by SiO_2, CH_2Cl_2, and ethyl acetate] to give 60 mg of **18**: IR (neat) 1675 cm^{-1} (C═O); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 7.4 Hz), 1.40−1.60 (m, 11 H), 1.68−1.71 (m, 4 H), 2.01−2.06 (m, 1 H), 2.45 (t, 2 H, J = 7.5 Hz), 2.51 (t, 2 H, J = 7.2 Hz), 2.58 (s, 4 H), 2.56−2.65 (m, 1 H), 2.68−2.75 (m, 1 H), 2.81−2.94 (m, 2 H), 2.98−3.03 (m, 1 H), 3.77 (t, 2 H, J = 7.2 Hz), 6.77−6.82 (m, 1 H), 6.84−6.87 (m, 1 H), 7.02−7.07 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 11.8, 22.1, 22.7 (d, $^3J_{C,F}$ = 3.0 Hz), 24.1 (2C:s), 24.9, 25.9, 26.4, 31.9, 37.5 (2C:s), 39.4 (2C:s), 44.9 (2C:s), 50.0, 52.4, 56.1, 111.7 (d, $^2J_{C,F}$ = 21.3 Hz), 123.9 (d, $^2J_{C,F}$ = 17.5 Hz), 124.7 (d, $^4J_{C,F}$ = 2.3 Hz), 126.4 (d, $^3J_{C,F}$ = 9.1 Hz), 139.5 (d, $^3J_{C,F}$ = 4.6 Hz), 160.8 (d, $^1J_{C,F}$ = 242.8 Hz), 172.1 (2C:s).

Pharmacology. [^{3}H]Raclopride Binding to Cloned DA D_2 and D_3 Receptors. Mouse fibroblast (Ltk^-) cells expressing human DA D_2 (long isoform) receptors were obtained from Dr. O. Civelli (Vollum Institute, Portland, OR), and chinese hamster ovary (CHO) cells expressing human DA D_3 receptors were obtained from INSERM Institute (Paris, France). The cells were grown and membranes prepared as described previously.[36] In brief, the cells were detached with 0.05% trypsin and 0.02% EDTA, collected by centrifugation, and homogenized in 10 mM Tris-HCl containing 5 mM $MgSO_4$. The homogenate was washed and stored in aliquots at −70 °C. On the day of the experiment the membranes were thawed, homogenized using an Ultra-Turrax, and suspended in binding buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 4 mM $MgCl_2$, and 1 mM EDTA, pH 7.4, to a final concentration of 5−25 μg of protein/0.5 mL. The competition experiments with 2 nM [^{3}H]raclopride (specific activity 74 Ci/mmol; DuPont New England Nuclear, Boston, MA) and test compound (10−12 concentrations) were performed at 30 °C for 60 min. Nonspecific binding was defined with 1 μM (+)-butaclamol (Research Biochemicals Inc., Natick, MA). The radioligand and the various compounds were dissolved in ascorbic acid (final concentration 0.01%). The incubations were terminated by rapid filtration through Whatman GF/B filters and subsequent washing with cold buffer (50 mM Tris-HCl, pH 7.4) using a cell harvester (Brandel). Scintillation cocktail (Packard Ultima Gold, 4 mL) was added, and the radioactivity was determined in a Packard 2500TR liquid scintillation counter at about 50% efficiency. The binding curves were analyzed by nonlinear regression using the LIGAND program.[52] The dissociation constants (K_d) of [^{3}H]raclopride, used to calculate the inhibition constants (K_i) of the various compounds, were 1.1 ± 0 nM (n = 3) for D_2 and 1.0 ± 0.1 nM (n = 3) for D_3 receptors.

[^{3}H]Quinpirole Binding to Cloned DA D_2 Receptors. The cells were grown and the membranes prepared as described for the [^{3}H]raclopride binding assay. On the day of the experiment the frozen membranes were thawed, homogenized using an Ultra-Turrax, and suspended in binding buffer containing 50 mM Tris-HCl, 120 mM *N*-methyl-D-glutamine, 5 mM KCl, 4 mM $MgCl_2$, and 1 mM EDTA, pH 7.4, to a final concentration of 250 μg of protein/2 mL. The competition experiments with 5−6 nM [^{3}H]quinpirole (specific activity 40 Ci/mmol; DuPont New England Nuclear, Boston, MA) and test compound (10−12 concentrations) were performed at 30 °C for 60 min. Nonspecific binding was defined with 1 μM (+)-butaclamol. The radioligand and the various compounds were dissolved in ascorbic acid (final concentration 0.01%). The incubation was terminated, the radioactivity was determined, and the binding curves were analyzed as described for the [^{3}H]-raclopride binding assay. The dissociation constant (K_d) of [^{3}H]-quinpirole, used to calculate the inhibition constants (K_i) of the various compounds, was 4.2 ± 0.7 nM (n = 3) for D_2 receptors.

[^{3}H]-8-OH-DPAT Binding to 5-HT_{1A} Receptors. Male Sprague−Dawley rats (weighing 150−220 g; B & K Universal AB, Sollentuna, Sweden) were decapitated, and the hippocampus was dissected out on ice. The tissue was homogenized in 50 mM Tris-HCl containing 10 mM EDTA (pH 7.4) using the Ultra-Turrax followed by centrifugation for 10 min at 48000*g* and 5 °C. The pellet was resuspended in 50 mM Tris-HCl and recentrifuged. The final pellet was frozen in 0.32 M sucrose and stored at −70 °C. On the day of the experiment the frozen homogenate was thawed, homogenized, and suspended in binding buffer containing 50 mM Tris-HCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$, pH 7.6, to a final concentration of 1.5 mg of original wet wt/2 mL. To remove endogenous serotonin the hippocampal membranes were preincubated for 10 min at 37 °C, and thereafter 10 μM pargyline was added. The competition experiments with 1−2 nM [^{3}H]-8-OH-DPAT (specific activity 154 Ci/mmol; DuPont New England Nuclear, Boston, MA) and test compound (10−12 concentrations) were performed at 30 °C for 60 min. Nonspecific binding was defined with 100 μM 5-HT (Sigma Chemical Co., St. Louis, MO). The radioligand and various compounds were dissolved in ascorbic acid (final concentration 0.01%). The incubation was terminated, the radioactivity was determined, and the binding curves were analyzed as described for the [^{3}H]-raclopride binding assay. The dissociation constant (K_d) of [^{3}H]-8-OH-DPAT for 5-HT_{1A} receptors, used to calculate the inhibition constants (K_i) of the various compounds, was 0.43 ± 0.05 nM (n = 4).

[^{35}S]GTPγS Binding to Cloned DA D_2 and D_3 Receptors. The cells were grown and the membranes prepared as described for the [^{3}H]raclopride binding assay. The [^{35}S]-GTPγS binding assay was performed as previously described[41−43] with some modifications. On the day of the experiment the frozen membranes were thawed, homogenized using an Ultra-Turrax, and suspended in the assay buffer containing 50 mM Tris-HCl, 100 mM NaCl, and 10 mM $MgCl_2$, pH 7.6, to a final concentration of 25 μg of protein/0.5 mL (D_2) and 16 μg/0.5 mL (D_3). The membranes, 1 μM GDP, and appropriate drugs were preincubated for 30 min at 30 °C in order to assure equilibrium before the addition of label; 100 pM [^{35}S]GTPγS (specific activity 1228 Ci/mmol; DuPont New England Nuclear, Boston, MA) was added, and the reaction was continued for 30 min at 30 °C. The incubation was terminated by rapid filtration through Whatman GF/B filters and subsequent washing with cold buffer containing 50 mM Tris-HCl and 10 mM $MgCl_2$, pH 7.4, using a cell harvester (Brandel). The radioactivity was determined in a Packard 2500TR liquid scintillation counter at about 100% efficiency.

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